The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes

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Two major 'seed' types occur in fynbos Proteaceae, those with nut-like, and those with winged or hairy achenes produced by serotinous species. Seeds of both types were soaked in 1% H_2O_2, an oxygenating treatment, or in water and germinated in open seed beds in autumn. Germination percentage in 13 of 15 serotinous species was not influenced by H_2O_2 treatment. In 10 out of 14 species with nut-like seeds, germination percentage was increased significantly, the mean increase being 89% of the control. Dormancy, imposed by the pericarp in species with nut-like seeds, is discussed in an ecological context.
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Twee hoof 'saad'-tipes kom in fynbos-Proteaceae voor: neutagtige, en gevlerkte of harige akene geproduseer deur serotiniese spesies. Sade van beide tipes is in 1% H_2O_2 geweek, as oksiginerende behandeling, of in water. Daarna is dit in oop saadbeddings in die herfs geplant om te ontkiem. Kiemingpersentasie van 13 uit 15 serotiniese spesies was nie deur H_2O_2-behandeling beinvloed nie. In die geval van neutagtige sade was die kiemingpersentasie van 10 uit 14 spesies betekenisvol verhoog. Gemiddeld oor spesies was die verhoging 89% van die kontrole. Saadrus, bepaal deur die perikarp in spesies met neutagtige sade, word in ekologiese verband bespreek.
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Introduction

Two major types of achenes ('seeds') are produced by sclerophyllous fynbos Proteaceae. These are, firstly, the winged or hairy seeds of serotinous species in which dispersal is delayed, usually until the parent plant is killed by fire, as in *Protea* (Bond 1984, 1985). Secondly, nut-like seeds which are dispersed annually after the flowering season, as in *Leucospermum* (Rourke 1972). Serotinous seeds germinate at or very near the soil surface (personal observation) after being dispersed by wind (Slingsby & Bond 1982). Nut-like seeds, on the other hand, are mostly myrmecochorous, i.e. they are dispersed and hoarded by ants in small subterranean nests (Slingsby & Bond 1982) at average depths varying between 3,0 to 4,5 cm in the immediate vicinity of parent plants (unpublished data). Apparently non-myrmecochorous nut-like seeds, such as in *Leucadendron* Section Leucadendron, are also buried in the soil (unpublished data).

The marked dormancy shown by especially the myrmecochorous Proteaceae (Slingsby & Bond 1981) presents a serious problem in both propagation from seed and in breeding. When the oxygen partial pressure in germinating seeds was raised by incubation in oxygen gas, the percentage germination increased in *Protea compacta* R.Br., *Leucadendron daphnoides* (Thunb.) Meisn. and *Leucospermum cordifolium* (Salisb. ex Knight) Fourcade (Brown & Van Staden 1973; Van Staden & Brown 1973).

Hydrogen peroxide could have an oxygenating effect when imbibed by achenes of Proteaceae species (Brown & Dix 1985). Soaking in hydrogen peroxide solutions was shown to be practical and economical as a one-time oxygenating treatment to obtain complete germination of viable seeds of *Leucospermum cordifolium* sown in open seed beds in autumn (Brits & Van Niekerk 1976). This treatment did not, however, improve the germination of *Serruria florida* Knight seeds incubated in petri dishes (Brits 1986). It therefore was necessary to investigate how effectively H_2O_2 can be used as a general treatment for improving the germination in different fynbos Proteaceae species.

Materials and Methods

Three experiments were conducted in 1977, 1979 and 1982 respectively, at Riviersonderend, Cape Province. Seeds of different Proteaceae species were used in each experiment. These were obtained either from the Department of Environmental Affairs or were collected personally in the wild or from cultivated stands. Hand sorted seeds of 15 serotinous species and 14 species with nut-like seeds (Table 1) were soaked for 24 h in either 1% H_2O_2 or water (control). After treatment

they were sown, in autumn, in standard seed beds for Proteaceae seed germination (Vogts 1976). In all experiments H_2O_2-treated and control seeds of each species were sown in paired plots within blocks, and irrigated daily. Newly germinated seeds were counted weekly for 20 weeks after sowing. In one experiment Chi-square tests for proportions were performed (Snedecor & Cochran 1967). In the other two experiments treatments were replicated in randomized blocks and ordinary least significant differences were calculated.

Results

The results for the three experiments are tabulated together in Table 1. In 13 of the 15 serotinous species tested, H_2O_2 treatment had no significant effect on germination percentage. The mean germination percentages realized for treated and untreated seeds differed by only 0,4%. In species with nut-like seeds, however, H_2O_2 treatment led to higher germination percentages in 12 of the 14 species tested, the differences being significant for 10 species. The mean increase in germination due to H_2O_2 treatment was 89% of the control. Individual increases were as high as 257% (*Leucospermum lineare*), 400% (*L. pluridens*) and 447% (*Leucadendron pubescens*).

Discussion

The results suggest that in most species with nut-like seeds, embryos have a strong requirement for increased oxygen partial pressure during germination. This requirement is generally absent in serotinous species. The distinction is drawn on the generic (*Aulax, Protea* vs. *Leucospermum, Serruria*) and sub-generic (*Leucadendron* Section Alatosperma vs. Section Leucadendron) levels. The present exceptions cannot be explained but in the case of species with nut-like seeds of low viability, incomplete pericarp development due to premature harvesting may have been responsible for a poor reaction to H_2O_2 treatment (Brits & Van Niekerk 1986). Other factors causing low mean germination in trials could possibly have been poor seed quality due to inadequate sorting, shrunken embryos (Van Staden & Brown 1973) and old seed.

It was suggested by Deall & Brown (1981) that diffusion of oxygen into seeds is not inhibited by the pericarp in *P. magnifica* Link, a serotinous species. The pericarp nevertheless prevents immediate germination of some seeds. This effect of the pericarp in serotinous species is, however, overridden by the strong promotive effect of low temperature (Deall & Brown 1981; Bond 1984). Dormancy in serotinous species therefore seems to be imposed physiologically in the embryo by low temperature requirement only, which in nature synchronizes germination with the first winter season following dispersal. The effect of the pericarp could be to prevent germination of the entire seed bank at one time, by extending germination over a substantial period of time (Deall & Brown 1981). This pattern of germination could have survival value in nature (Mayer & Poljakoff-Mayber 1982).

Although incubation in oxygen gas increased the germination percentage of seeds of *Protea compacta*, another serotinous species, the effect of oxygen on germination of the nut-like seeds of *Leucadendron daphnoides* was much stronger (Brown & Van Staden 1973). This partially confirms the present results, which indicate pericarp-imposed dormancy by means of restricted oxygen passage, only in species with nut-like seeds. It seems likely, therefore, that the pericarp in species with nut-like seeds, which are buried in nature, would have an important ecophysiological function which is absent in serotinous species.

The availability of oxygen may be reduced or the O_2/CO_2 ratio of the gaseous phase in the soil may be changed by the presence of both appreciable vegetation and many micro-organisms (Mayer & Poljakoff-Mayber 1982). The overall density of plants in fynbos is relatively very high (Kruger 1979). Proteaceous plants in mature fynbos develop, during winter, a distinct layer of proteoid roots in the uppermost 50 mm of soil. These ephemeral roots, which contribute up to 80% of the dry weight of the total root system, are replaced entirely each winter (Lamont 1983). During winter, microbial

Table 1 Influence of H_2O_2 oxygenating treatment on the mean germination percentage of 29 species in 5 genera of Proteaceae with either serotinous (s) or nut-like (n) seeds

Taxon	Seed type	Control[b] (water)	H_2O_2[b]	Difference
Aulax Berg.				
Aulax cancellata (L.) Druce	s	14,7	16,0	ns
Leucadendron R.Br. (Section Alatosperma)				
L. discolor Phill. & Hutch.	s	55,2	68,3	**
L. eucalyptifolium Beuk ex Meisn.	s	79,3	74,0	ns
L. muirii Phill.	s	34,1	38,8	ns
L. nobile Williams	s	53,3	50,5	ns
L. rubrum Burm.	s	93,8	71,4	ns
L. uliginosum R.Br.	s	49,2	48,5	ns
Protea L.				
P. caffra Meisn.[a]	s?	8,4	15,0	*
P. compacta R.Br.	s	11,3	9,7	ns
P. cynaroides (L.) L.	s	33,0	35,7	ns
P. effusa E. Mey. ex Meisn.	s	14,8	16,7	ns
P. punctata Meisn.	s	47,6	45,0	ns
P. repens (L.) L.	s	24,0	22,0	ns
P. speciosa (L.) L.	s	36,0	37,5	ns
P. subvestita N.E. Br.	s	42,2	42,1	ns
Mean		39,8	39,4	
Leucadendron R.Br. (Section Leucadendron)				
L. daphnoides (Thunb.) Meisn.	n	0,0	10,7	**
L. pubescens R.Br.	n	7,6	41,4	**
L. sessile R.Br.	n	28,9	44,9	**
L. tinctum Williams	n	0,0	12,0	**
Leucospermum R.Br.				
L. cordifolium (Salisb. ex Knight) Fourcade	n	16,0	43,1	**
L. cuneiforme (Burm. f.) Rourke	n	13,8	16,0	ns
L. fulgens Rourke	n	12,2	31,1	*
L. glabrum Phill.	n	24,3	49,8	**
L. lineare R.Br.	n	16,7	59,7	**
L. patersonii Phill.	n	2,7	17,0	**
L. pluridens Rourke	n	12,5	62,5	**
L. praemorsum (Meisn.) Phill.	n	66,7	61,9	ns
Serruria Salisb.				
S. florida Knight	n	12,0	16,3	ns
S. rosea Phill.	n	58,6	46,9	ns
Mean		19,4	36,7	

* = significant at $P=0,05$; ** = significant at $P=0,01$; and ns = not significant

[a]A weakly serotinous non-fynbos species of the summer rainfall region

[b]Seeds were soaked in 1% H_2O_2 or water for 24 h.

activity would be high in both the decaying mass of old proteoid roots and the substantial wet leaf litter layer, which accumulate under Proteaceae and other fynbos plants after the annual leaf-fall in late summer and autumn (Bond 1980; Pierce & Cowling 1984). The oxygen partial pressure in ant nests where seeds are hoarded may presumably be reduced by respiration.

During the winter germination season (Brits & Van Niekerk 1986), one or a combination of the above factors may conceivably reduce the availability of oxygen in the soil to a suboptimal level in unburnt fynbos. The soil aeration would improve after a fire, since all mature vegetation is destroyed (Kruger 1979) and since subterranean chambers where seeds are hoarded by ants are apparently abandoned by them (unpublished data). It is suggested that with nut-like seeds, which remain dormant in the soil for extended periods in unburnt fynbos, an improved oxygen status in the soil after fire may act as a germination cue.

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